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(54) Title: METHOD FOR SCREENING THE RISK OF GASTRIC CANCER

## (57) Abstract

Method for screening the risk for gastric cancer using, in combination, the determination of serum pepsinogen 1, gastrin-17 and the supporting determination of *Helicobacter pylori* antibodies from blood serum, in order to detect either atrophy of the corpus area, atrophy of the antrum area or atrophy of the mucosa of the whole stomach as well as a causative *Helicobacter pylori* infection, whereby the risk for gastric cancer can be evaluated and the necessary gastroscopy and follow-up can be planned.

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**Method for screening the risk of gastric cancer**

**Background of the invention**

5

In the following background information is presented relating to methods for screening the risk of gastric cancer, primarily using pepsinogen I and gastrin-17 determination from a blood sample.

10

Although the occurrence of new cases of gastric cancer has diminished in the recent years, gastric cancer is still one of the most common malignancies. In Finland, appr. 250 to 300 new cases of cancer/one million people/year are registered. In the age group of people above 50, there are an estimated 2350 cases of stomach cancer, which is about 3 per mille of the age group population (Finnish Cancer Registry - The Institute for Statistical and Epidemiological Cancer Research 1993). In addition to Finland, there is a high gastric cancer incidence in Iceland, South America and especially in Japan.

The prognosis of gastric cancer is usually poor, as there is no specific treatment. Presently the only possibility 25 of successfully treating gastric cancer is its early detection and total removal surgically.

Gastric cancer does not necessarily give any symptoms in 30 its early stages. The late appearance of symptoms naturally delays the patient from seeking treatment. On the other hand, the clinical findings in the early stage of gastric cancer are often non-specific. The primary diagnostic method for gastric cancer is presently gastroscopy and biopsies, cell and aspiration cytology associated 35 therewith. As routine gastroscopies are carried out in order to examine symptoms, such as pain in the upper abdomen or bleeding of the gastrointestinal tract, a symptomatic gastric cancer discovered in this manner is often already far advanced and thus inoperable. Attempts

have also been made at improving primary diagnostics with various immunological methods, but no sufficiently specific immunological method has been successfully developed.

- 5 It is a primary object to find the means by which it would be possible to identify within the general population easily and with moderate costs those symptomless persons which might be suffering from gastric cancer in its initial stages. After identification these persons
- 10 should immediately be examined by gastroscopy. At the same time those persons could be identified which exhibit premalignant gastric changes which need to be followed up.
- 15 Gastric cancer can be preceded by a number of different gastric diseases or conditions (so called precancerous conditions), which are chronic atrophic gastritis, pernicious anaemia, ventricular ulcer, gastric polyposis and the Ménétrier disease (giant hypertrophic gastritis).
- 20 Clearly identifiable changes of the mucosa are dysplasia and adenoma. The said conditions are associated with an appr. 4 to 5 fold relative cancer risk, as compared to the general population. It has been established that in almost all diseases the risk is mediated over chronic at-
- 25 rophic gastritis.

- 30 Chronic gastritis means a prolonged inflammatory condition of the gastric mucosa. The disease can coarsely be divided into the so-called superficial and the atrophic form. In superficial gastritis, the inflammatory cell infiltration is concentrated below the surface epithelium. In case the inflammation progresses and diffuses between the specific gastric secretory glands, one refers to chronic atrophic gastritis. In such a case, the normal glandular structures of the gastric mucosa are at least partly substituted by metaplastic changes.
- 35

The relative risk of gastric cancer in patients suffering from atrophic gastritis in the corpus area of the stomach, has been estimated, as calculated from the Finnish cancer statistics, to be about 4- to 5-fold as compared to persons having a healthy mucosa. In addition, there is a risk for falling ill with pernicious anaemia due to intrinsic factor deficiency and B12 vitamin absorption disturbance. In severe atrophy of the antrum area, the risk is even 18-fold. If atrophic changes appear both in the antrum and the corpus area (pangastritis), the risk can increase to even 90-fold (Sipponen, Kekki, Haapakoski, Ihmäki & Siurala 1985).

15 Screening for preliminary stages of gastric cancer by determination of pepsinogen and gastrin-17 in serum

*Stomach pepsinogens*

It is possible to electrophoretically distinguish 7 different pepsinogens from the gastric mucosa in humans (Samloff 1969). Of these the five fastest form the immunologically uniform group of pepsinogen I. The other two form the pepsinogen II group. The group I pepsinogens are synthesized only in the main cells and the mucous secreting cells of the corpus area of the stomach. In contrast thereto, group II pepsinogens are formed in the glands over the whole stomach area and to some degree also in the upper part of the duodenum in the Brunner glands (Samloff & Liebman 1973; Weinstein, Lechango, Samloff et al 1977). In the serum of a healthy person the pepsinogen I concentration is approximately 6 times that of the pepsinogen II concentration (Samloff 1982). In atrophic gastritis of the corpus area of the stomach the serum pepsinogen I concentration decreases, whereas the serum pepsinogen II concentration remains at the previous level. Thus, the serum pepsinogen I concentration fairly well reflects the number of pepsinogen secreting cells in

the corpus area of the stomach, and their condition. The more serious the atrophic gastritis of the corpus area of the stomach is, the lower is the serum pepsinogen I concentration (Tamm, Villako, Härkönen & Karonen 1984;

5 Kekki, Samloff, Varis & Ihamäki 1991). A low pepsinogen I concentration in the serum indicates severe atrophic corpus gastritis with a sensitivity of over 90 % and a specificity of almost 100 % (Varis, Kekki, Härkönen, Sipponen & Samloff 1991).

10

In a Finnish study (Varis, Sipponen, Laxén, Härkönen & Heinonen, still unpublished) wherein symptom-free smoking men over the age of 50 were screened for gastroscopical examination based on a low serum pepsinogen I concentra-

15 tion, a neoplastic change was observed in 4.7 % of those that had undergone gastroscopy (5.8 % of the patients suffering from atrophic gastritis) and in 1 % a symptom-free precancerous condition (Table 1 and Table 2) was observed. In this study it was possible to identify, ba-

20 sed on the low serum pepsinogen I test result, a number of gastric cancer incidences requiring immediate surgery and pre-cancerous conditions (dysplasia) requiring subsequent gastroscopic following. At the same time it was possible to identify the population group which runs the

25 risk of falling ill with pernicious anaemia and whose blood values should consequently be monitored in the future.

Epidemiological studies show that only about 25 % of 30 gastric cancer patients have severe atrophic corpus gastritis which can be verified based on a low serum pepsinogen I level (Sipponen, Kekki, Haapakoski; Ihamäki & Siurala 1985). In about 65 % the cancer is preceded by atrophy in the antrum area, which thus is not verifiable by pepsino-  
35 gen I determination.

**Table 1.**

5      Results from a study for screening gastric cancer in Finnish smoking men over the age of 50, based on a low serum pepsinogen I (S-PG I) level

	Screening result	Number	% of the former	% of those gastroscopied
10	Number examined	22431		
	Low S-PG I	2215	9.9	
	Gastroscopied	1347	60.8	
	Atrophic corpus			
15	gastritis	1092	81.1	
	Carcinoma	63	5.8	4.7

**Table 2.**

20      Neoplastic (tumour like) changes of the gastric mucosa based on pepsinogen I determination in men over 50 years of age examined in Finland (see Table 1).

	Neoplastic type	Number
25	Moderate dysplasia (abnormal growth)	42
	Severe dysplasia	7
	Carcinoma	11 (initial 70%)
	Carcinoid tumour	3
30	Total number	63

**Gastrin of the antrum area**

35      Gastrin is secreted in the gastrointestinal tract in at least three different forms, the immunoreactive activity of all these forms being measured when serum gastrin is

determined (total serum gastrin). Gastrin subtypes are the so-called minigastrin (G-14), little gastrin (G-17) and big gastrin (G-34) (Gregory 1974). Physiologically most important are gastrin-17 and gastrin-34. The effect  
5 of gastrin-17 on the secretion of hydrochloric acid is 6 times that of gastrin-34 (Walsh, Isenberg, Ansfield & Maxwell 1976). Gastrin is secreted from the so-called G-cells, which appear both in antrum and in duodenum. The most important accelerators of gastrin secretion is the  
10 tonus of the vagus nerve and the protein degradation products. The secretion of gastrin is slowed down by a pH decrease of below 2.5 (Walsh, Richardson & Fordtran 1975). The gastrin secreted from the antrum is to over 90 % of the gastrin-17 type, whereas the duodenal gastrin  
15 is primarily of the gastrin-34 type (Berson & Yalow 1971). In a fasting situation, primarily gastrin-34 is found in the serum, whereas after a meal the serum gastrin is of the gastrin-17 type (Lamers, Harrison, Ippoliti & Walsh 1979). The secretion of gastrin-17 can also be  
20 studied using the so-called protein stimulation test. In such a test, a blood sample after fasting is taken in the morning, whereafter the patient eats a protein rich standard meal and blood samples are taken at 15 minute intervals for two hours. The maximal increase is evident after  
25 appr. 20 minutes.

In atrophic antrum gastritis the mucous membrane of the antrum is atrophied and thus its gastrin-17 secretion decreases and its concentration in the serum is reduced.  
30 A reduced gastrin-17 concentration in the serum would thus be an indicator of antrum atrophy and of an increased risk for cancer in this area. In case the mucous membrane of the antrum is atrophied, there is a reduced response also in the protein stimulation test, which seems  
35 to be a more sensitive indicator of atrophy than the mere concentration determination.

*Helicobacter pylori infection of the stomach*

5      *Helicobacter pylori* is a spiral shaped, gram-negative bacterium which thrives in the mucus in the immediate  
10     vicinity of the surface epithelial cells of the gastric mucosa and in the cell interstices. The bacterium apparently is transferred perorally from one person to the other. The effect of the bacterium on the gastric mucosa is an inflammation reaction, which is mediated over a complement by liberating strong inflammation mediator substances. After the acute stage, the inflammation is transformed into chronic gastritis. In patients suffering  
15     from chronic gastritis, in 70 to 90 % a *Helicobacter pylori* infection can be established (Calam 1994). As *Helicobacter pylori* infection and chronic gastritis in the stomach are closely associated, it has been stipulated that this bacterial infection could be one etiological factor in the development of stomach cancer. It is for this reason possible that eradication of the *Helicobacter pylori* bacteria in the initial stages of the infection,  
20     could prevent the development of atrophy associated with chronic gastritis, and thus reduce the cancer risk.

25     *Screening for gastric cancer using combined pepsinogen I - gastrin-17 - Helicobacter pylori-antibody determination in serum*

30     For the screening preferably a method can be used, according to which on a microplate simultaneously the serum pepsinogen I and gastrin-17 concentration and the *Helicobacter pylori* antibodies can be determined. It is hereby possible to diagnose either corpus gastritis, antrum gastritis or pangastritis, as well as one cause of atrophy, *Helicobacter pylori* infection. It is to be noted  
35     that in severe atrophic gastritis, no *Helicobacter pylori* bacteria can be found in the gastric mucosa, and also the

antibodies in the serum have been normalized.

For pepsinogen I determination the pepsinogen I antigen has been purified from the human gastric mucosa. It has  
5 been coupled by thiolation and protein-protein-conjugation to rabbit or sheep red blood cells and so rabbits or sheep have been immunized in the classical manner to produce antibodies to pepsinogen I. A polyclonal antibody to gastrin-17 has been produced with the  
10 same technique by coupling a [Leu<sup>15</sup>]-gastrin-17 (Sigma S-9145, [Leu<sup>15</sup>]-gastrin-I) or the gastrin-17 fragment 1-13 (Sigma G-6261) to rabbit autologous red blood cells and immunizing the rabbits in a normal manner the rabbits to produce a specific antibody to gastrin-17. Polyclonal antibodies have been produced also with BSA-conjugates in  
15 the classical manner. A monoclonal gastrin-17 antibody has been produced in the classical manner in mouse with a [Leu<sup>15</sup>]-gastrin-17-derivative, which is conjugated to thyroglobulin. The specific antibodies have been purified  
20 with protein A-affinity chromatography or in some cases with pepsinogen I or gastrin-17 affinity chromatography.

Pepsinogen I determination

- 25 1. The microplate wells are coated with a polyclonal antibody by incubating over night at + 4°C.
2. The wells are emptied and washed with a washing solution.
3. The sample is added at a suitable dilution, and incubated at the most one hour at + 37°C.
- 30 4. The wells are washed.
5. The enzyme labelled monoclonal antibody is added at a suitable dilution, and incubated for at the most one hour at +37 °C.
- 35 6. The wells are washed.
7. The substrate is added (chromogenic, fluorescent or luminescent substrate), and incubated maximally for 30

mins at room temperature.

8. The reaction is stopped and the absorbance, fluorescence or luminescence is determined and compared to a calibration curve.
- 5 The method can be speeded up by incubating the sample and the enzyme labelled monoclonal antibody simultaneously as a so-called one-step-ELISA-method. There are also other alternatives that can be used.

10 Alternative 2 (competitive ELISA)

1. The sample and antibody are incubated on microplate wells, whereby the antigen of the sample couples to the antibody.
- 15 2. A known amount of labelled antigen is added, which competes with the antigen in the specimen for free antibody.
3. The solution is transferred to a microplate well which has been coated with a second antibody, and incubated maximally for 1 hour.
- 20 4. Unbound material is washed away.
5. The substrate is added and incubated maximally for 30 minutes at room temperature.
6. The reaction is stopped and the results are evaluated
- 25 by comparing to calibration curve.

As an alternative, the antigens can be added simultaneously and/or directly to a microplate well coated with a second antibody, but in such a case the sensitivity of the process is not as good. In this process, the competition can be arranged also with labelled antibody.

Gastrin-17 determination

- 35 1. The wells are coated with streptavidin.
2. An excess of gastrin-17 derivative labelled with biotin is added and incubated until equilibrium is

reached.

3. The sample and the monoclonal antibody are added whereby the gastrin-17 in the specimen and the biotin labelled gastrin-17 compete for the antibody. The mixture

5 is incubated for one hour at + 37°C.

4. The wells are washed.

5. The enzyme labelled second antibody is added and incubated for appr. one hour.

6. The substrate (fluorescent or luminescent) is added

10 and incubated for appr. 30 minutes at room temperature.

7. The reaction is stopped and the fluorescence or luminescence is read, and compared to a calibration curve.

#### *Helicobacter pylori determination*

15

For the determination of the *Helicobacter pylori* antibodies a number of commercial "kits" are available (e.g. Orion Pyloriset EIA-G, Pyloriset EIA-A, EIA 2G by Roche, Pyloristat by Whittaker Bioproducts). Antigens can be

20 prepared from *Helicobacter pylori* bacteria in various ways (see Lelwala-Guruge, Nilsson, Ljungh, Wadström 1992) and they are also commercially available. The method we use for determining *Helicobacter pylori* antibodies is generally known and the antigen extracted from the bacte-

25 ria with acidic glycine is attached by adsorption to the wells in a microplate. The second antibody (rabbit anti-human IgG) is labelled with enzyme (alkaline phosphatase and in some cases peroxidase). p-Nitrophenyl phosphate (adsorbance) or umbelliferyl-phosphate (fluorescence) is

30 used as the enzyme substrate, whereby the absorbance (405 nm) or fluorescence (360 nm/460 nm) can be measured.

#### *Sensitivity of measurement*

35 In the determination of serum pepsinogen I the cut-off-value for atrophic gastritis is, according to our previous studies, 20-30 µg/l depending on the specificity

and sensitivity agreed upon for the method in question, which corresponds to appr. 450 - 690 pmol/l (Varis, Sipponen, Laxén, Härkönen & Heinonen, still unpublished). In a normal situation the gastrin-17 concentrations are in  
5 the range of 2 - 25 pmol/l. Thus, the serum gastrin-17 concentration is appr. 100 times smaller than that of pepsinogen I. As one measures even lower values in atrophic antrum gastritis, a very high sensitivity of the developed method is required. In atrophy of the antrum  
10 area the cut-off value for serum gastrin-17 determination is in the range of 0.1 - 2 pmol/l. It is possible to reach a sufficiently high sensitivity with an enzyme label and using, for example, luminescence measurement as a sensitive detection system. The sensitivity can be further increased by performing a so-called enzymatic recycling reaction which we have applied i.a. for the determination of steroids (Härkönen, Adlercreutz & Groman  
15 1974). Also in this case the product formed can be measured either using fluorescence technique or luminescence.  
20 For the *Helicobacter pylori* positiveness the cut-off titer is 200 - 500. The use of the combination method for detecting atrophy of the mucosa in the various parts of the stomach is shown in the Table 3.

25

30

35

Table 3. Combination method for serum pepsinogen I and gastrin-17 for detecting severe atrophic gastritis of the mucosa of the corpus area or the antrum area of the stomach, or of the whole stomach (pangastritis).

	Location of severe atrophy in stomach	Serum pepsino- gen I µg/l	Serum gastrin- 17 pmol/l	Increase of of serum gastrin- 17 (response) in protein stimula- tion
5				
10	Corpus	< cut-off value	> upper reference limit	normal or in- creased response
15	Antrum	> cut-off value	< cut-off value	strongly reduced response
20	Mucosa of the whole stomach (corpus and antrum)	< cut-off value	at the lower limit of reference value	reduced response
25	Reference values:	Serum pepsinogen I Serum gastrin-17	25 - 120 µg/l 2 - 25 pmol/l	
30	Cut-off-values:	Serum pepsinogen I Serum gastrin-17	20 - 30 µg/l 0.1 - 2 pmol/l	

According to the Table 3, when the serum pepsinogen I concentration is below the cut-off value, which according to the specificity of the method is in the range of appr. 35 20 - 30 µg/l, the atrophy is located in the corpus area of the stomach. If, on the other hand, the serum gastrin-17 concentration is below the cut-off value, which depen-

ding on the specificity of the method is in the range of 0.1 - 2 pmol/l, the atrophy is located in the antrum area of the stomach. In atrophy of the corpus area the gastrin-17 value is above the upper limit of the reference values. Correspondingly in pangastritis, the serum pepsinogen I concentration is below the cut-off value whereas the gastrin-17 concentration is at the lower limit of the reference values. The location of the atrophy can also be verified using the protein stimulation test, in which the serum gastrin-17 concentration is measured at the base line situation and after a protein rich standard meal. The response (increase of serum gastrin-17 as compared to the base line situation) is strongly reduced in atrophy of the antrum area only, and slightly decreased in atrophy of the mucosa of the whole stomach. On the other hand, if the mucosa of the antrum is healthy, but the corpus area is atrophied, the response is normal or increased.

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## Claims

1. Method for screening the risk for gastric cancer from blood serum by detecting atrophy of the corpus or antrum area of the stomach, or atrophy of the mucosa of the whole stomach, characterized in that the pepsinogen I and gastrin-17 concentrations are determined quantitatively from the serum and the values obtained are compared to the method specific cut-off value for the substance to be determined.
- 5
- 10
2. Method according to the claim 1, characterized in that the pepsinogen I and gastrin-17 concentration values obtained are compared also to the reference value of the substance to be determined.
- 15
3. Method according to the claim 1 or 2, characterized in that the serum gastrin-17 concentration is also measured using the protein stimulation test by measuring the said concentration at the base line situation and after a protein rich standard meal.
- 20
- 25
4. Method according to the claim 1, 2 or 3, characterized in that a combined immunological pepsinogen I and gastrin-17 method is used on a plastic, glass or cellulose support.
- 30
5. Method according to the claim 4, characterized in that the support is a microplate well.
- 35
6. Method according to any one of the claims 1 to 5, characterized in that a detection method for absorbance, fluorescence or luminescence is used for the pepsinogen I and gastrin-17 determination.
7. Method according to any one of the claims 4 to 6, characterized in that for the determination of the pepsino-

gen I concentration, a polyclonal or monoclonal antibody to pepsinogen I is used.

5       8. Method according to any one of the claims 4 to 7, characterized in that for the determination of the gastrin-17 concentration, specific poly- or monoclonal antibody to gastrin-17 are used.

10      9. Method according to the claim 8, characterized in that a polyclonal antibody to gastrin-17 is obtained by immunizing an animal, such as a rabbit or a lamb, with the gastrin fragment 1-13, [Leu<sup>15</sup>]-gastrin-17 or using a gastrin-17 antigen isolated from the stomach of an animal such as a pig.

15      10. Method according to the claim 8, characterized in that a monoclonal antibody has been produced in a mouse using the [Leu<sup>15</sup>]-gastrin-17 antigen.

20      11. Method according to any preceding claim, characterized in that is combined with a *Helicobacter pylori* antibody determination.

25      12. Method according to any preceding claim, characterized in that the pepsinogen I, gastrin-17 and *Helicobacter pylori* determination methods are combined to a kit-method, wherein the determinations are carried out simultaneously on a microplate well using a method based on the ELISA technique, using polyclonal and monoclonal antibodies, as well as streptavidin-biotin-interaction, as well as sensitive detection methods based on absorption, fluorescence or luminescence.

1  
INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 95/00634

**A. CLASSIFICATION OF SUBJECT MATTER****IPC6: G01N 33/74, G01N 33/573**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

**IPC6: G01N**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

**SE,DK,FI,NO classes as above**

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**MEDLINE, BIOSIS, WPI****C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Gastroenterol, Volume 26, No 186, 1991, K. Varis et al, "Serum Pepsinogen I and Serum Gastrin in the Screening of Atrophic Pangastritis with High Risk of Gastric Cancer", page 117 - page 123, See "Discussion"  --	1-9,11,12
X	Scand J Gastroenterol, Volume 26, No 186, 1991, M. Kekki et al, "Serum Pepsinogen I and Serum Gastrin in the Screening of Severe Atrophic Corpus Gastritis" page 109 - page 116  --	1-9,11,12
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 Further documents are listed in the continuation of Box C. See patent family annex.

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20 February 1996	21 -02- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Authorized officer  Carl-Olof Gustafsson Telephone No. + 46 8 782 25 00

## INTERNATIONAL SEARCH REPORT

International application No.

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## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Dialog Information Service, file 154, Medline, Dialog accession no. 07951582, Medline accession no. 92089582, Farinati F. et al: "Pepsinogen A/pepsinogen C or pepsinogen A multiplied by gastrin in the diagnosis of gastric cancer", Ital J Gastroenterol (ITALY) May 1991, 23 (4) p194-6  --	1-9,11,12
A	Digestive Diseases and Sciences, Volume 29, No 9, Sept 1984, Haruka Sasaki et al, "Low Acid Output in Pima Indians A Possible Cause for the Rarity of Duodenal Ulcer in this Population" page 785 - page 789  --	1-9,11,12
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A	Dialog Information Service, file 154, Medline, Dialog accession no. 08512746, Medline accession no. 93222746, Sitas F. et al: "Serum anti- Helicobacter pylori IgG antibodies and pepsinogens A and C as serological markers of chronic atrophic gastritis", Cancer Epidemiol Biomarkers Prev 1993, 2 (2) p119-23  --	11,12
A	Clin.Chem., Volume 31, No 1, 1985, Göran Lindstedt et al, "Analytical and Clinical Evaluation of a Radioimmunoassay for Gastrin"  --	1-10

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Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Int.J. Cancer, Volume 35, 1985, Pentti Sipponen et al, "Gastric Cancer Risk in Chronic Atrophic Gastritis: Statistical Calculations of Cross-Sectional Data" page 173 - page 177 --	1
A	Clinica Chimica Acta, Volume 162, 1987, Shih Che Huang et al, "Enzyme-linked immunosorbent assay of serum pepsinogen I" page 85 - page 96 --	4-9
A	Scand J Clin Lab Invest, Volume 47, 1987, Gerard Pals et al, "Enzyme-linked immunosorbent assay and radioimmunoassay of serum pepsinogen A" page 29 - page 33 --	4-9
A	Clinica Chimica Acta, Volume 175, 1988, Shih Che Huang et al, "Enzyme-linked immunosorbent assays for serum pepsinogens I and II using monoclonal antibodies - with data on peptic ulcer and gastric cancer" page 37 - page 50 -- -----	4-9